

## **SUPPLEMENTARY FIGURES 1-19**

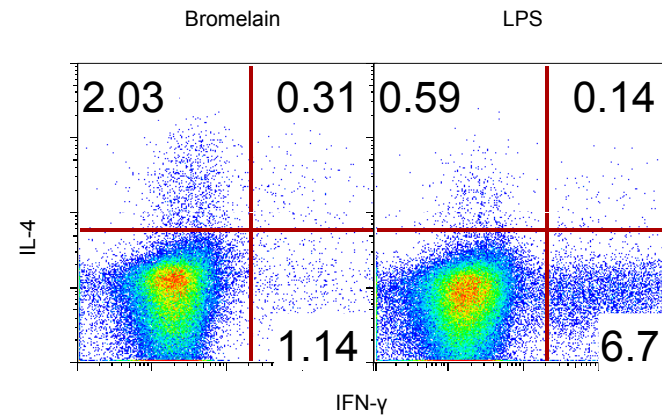
### **T<sub>H</sub>2 response to cysteine-proteases requires dendritic cell-basophil cooperation via ROS mediated signaling**

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#### **Reference**

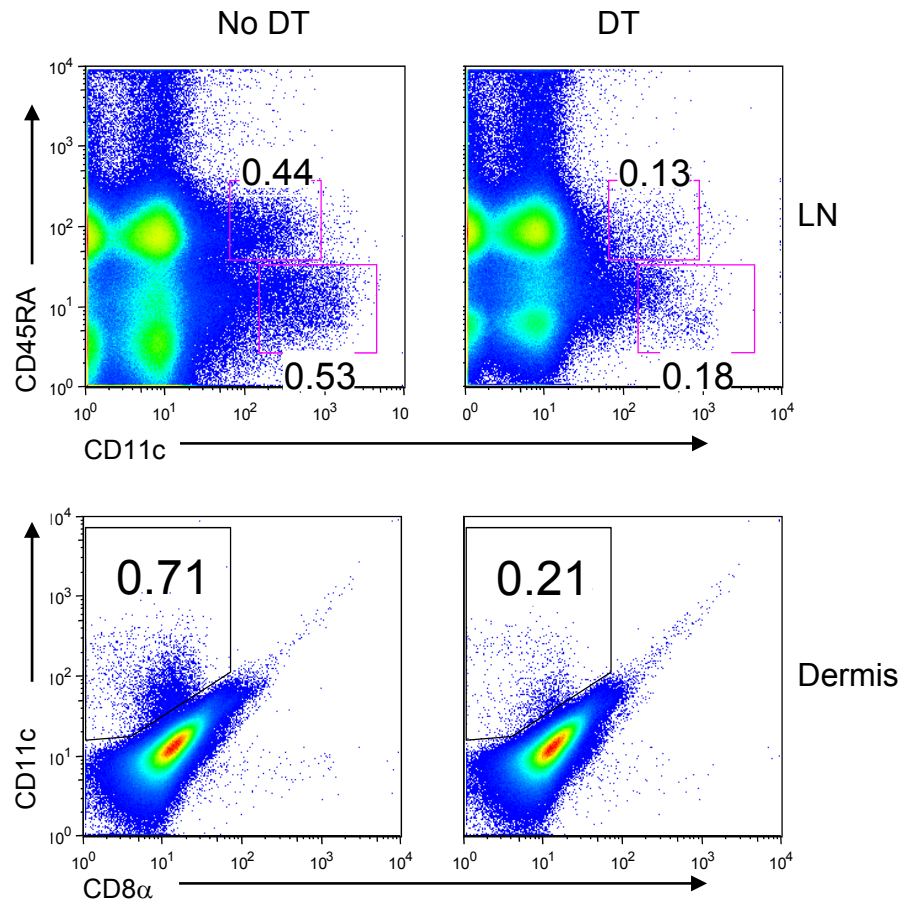
1. Pape, K.A., Catron, D.M., Itano, A.A. & Jenkins, M.K. The humoral immune response is initiated in lymph nodes by B cells that acquire soluble antigen directly in the follicles. *Immunity* **26**, 491-502 (2007).

## S1. Bromelain induces Th2 response



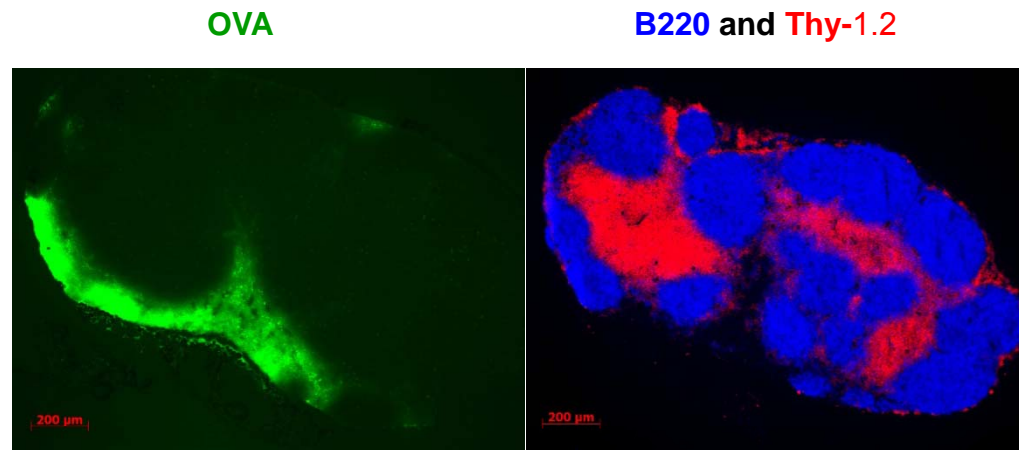
C57BL/6 mice were immunized subcutaneously with bromelain or LPS (50  $\mu$ g/mouse) plus OVA (100  $\mu$ g/mouse), and repeatedly boosted on day 7 and day 14. On day 21 after the primary immunization, the draining LNs were removed and total LN cells cultured in the presence of OVA in vitro for 5 days, and the cytokines IL-4/IFN- $\gamma$  produced by CD4<sup>+</sup> T cells were analyzed by intracellular FACS staining.

## S2. DCs can be efficiently depleted in CD11c-DTR mice by diphtheria toxin treatment



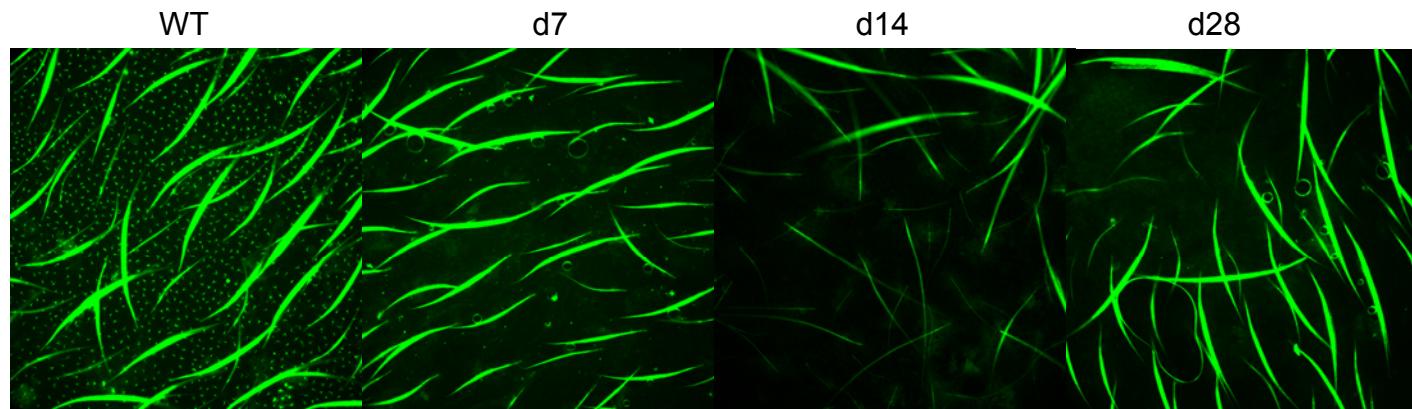
DCs can be efficiently depleted in CD11c-DTR mice by diphtheria toxin (DT) treatment. CD11c-DTR mice were intraperitoneally injected with PBS or 100 ng DT. 24 hr later, skin lymph nodes (LN) and ears were removed and single cells were prepared as described in *Methods*. The efficiency of depletion was analyzed by FACS. CD11c<sup>+</sup>CD45RA<sup>-</sup> is conventional DC CD11c<sup>low</sup>CD45RA<sup>+</sup> is plasmacytoid DC.

### S3. OVA reaches draining lymph node within two hours of subcutaneous injection



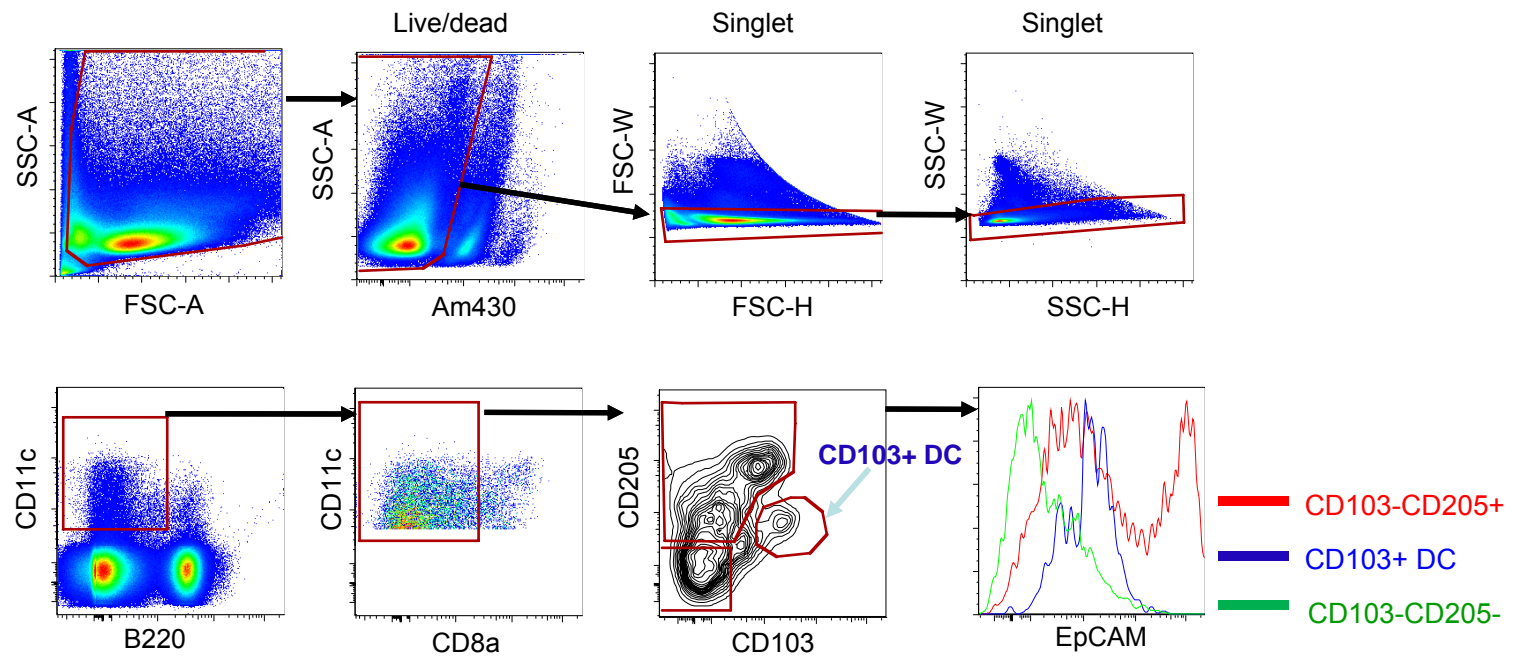
Soluble Alexa-488 labeled OVA (100 µg, green) mixed with unlabeled papain (50 µg) was injected subcutaneously. Two hours later the draining lymph nodes were isolated, frozen and cut into sections (as described in *Methods*). B220-Alexa647 (blue) and Thy1.2-PE (red) were used to stain B cells and T cells respectively, as described in Ref 1 in Supplementary Material.

S4. Langerhans cell can be depleted by injection of diphtheria toxin into Langerin-DTR mice



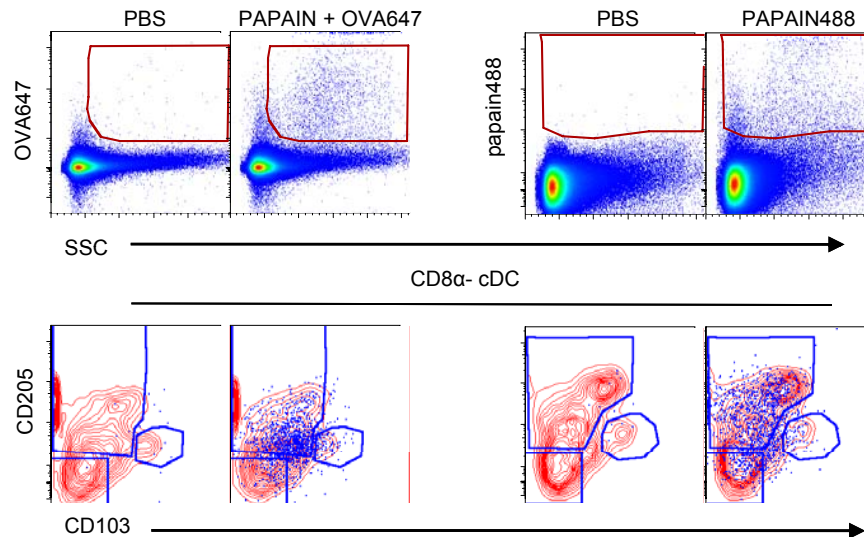
Langerhans cell in epidermis can be efficiently depleted by diphtheria toxin (DT) treatment of langerin-DTR mice. Wild type or langerin-DTR mice were intraperitoneally injected with 1  $\mu$ g DT. At the indicated time points, ears were removed and the epidermal sheets were prepared as described in *Methods*. The presence of Langerhans cells was analyzed by immunofluorescence of epidermal sheets by using antibody against IA/IE. Magnification, x10.

## S5. Identification of CD103<sup>+</sup> DCs in skin lymph node



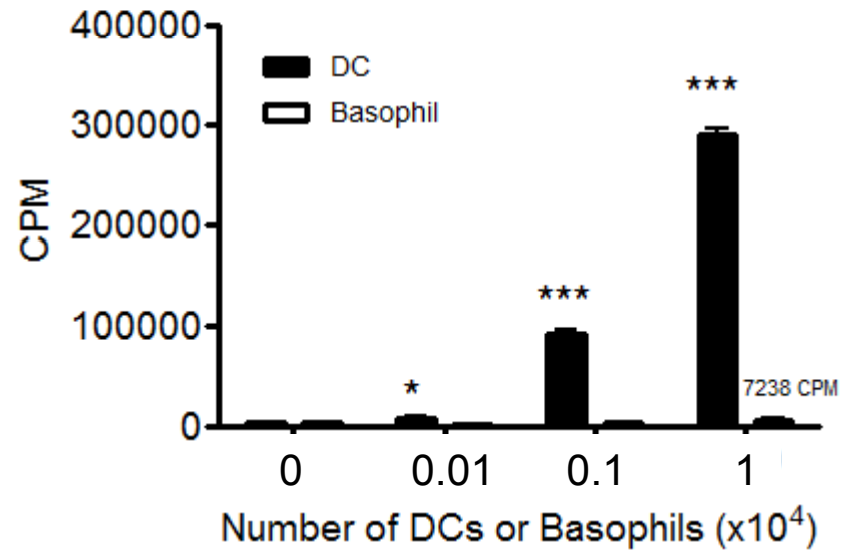
Lymph node cells were stained with the indicated antibodies and analyzed by FACS LSR II. Dead cells were excluded by Am430 dye and doublets were excluded, and singlets included as shown. CD103<sup>+</sup> DCs were identified as CD11c<sup>+</sup>CD8α<sup>-</sup>CD205<sup>+</sup>CD103<sup>+</sup>. The expression of the Langerhans cell marker EpCAM, by the different subpopulation is shown in the histogram.

S6. CD103<sup>+</sup> DCs do not efficiently take up antigen in response to immunization with OVA + papain



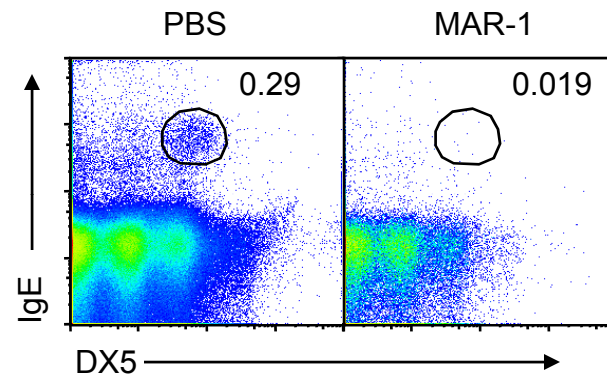
Analysis of uptake of fluorescently labeled OVA and papain by DC subsets as described in *Methods*. Gating strategy for DC subsets is described in Fig. S5. Papain-Alexa488<sup>+</sup> or OVA-Alexa647<sup>+</sup> cells were overlaid onto CD8α<sup>+</sup>cDC subsets defined in Fig. S5.

S7. DCs are much more efficient than basophils, in presenting protein antigen to CD4+ T cells



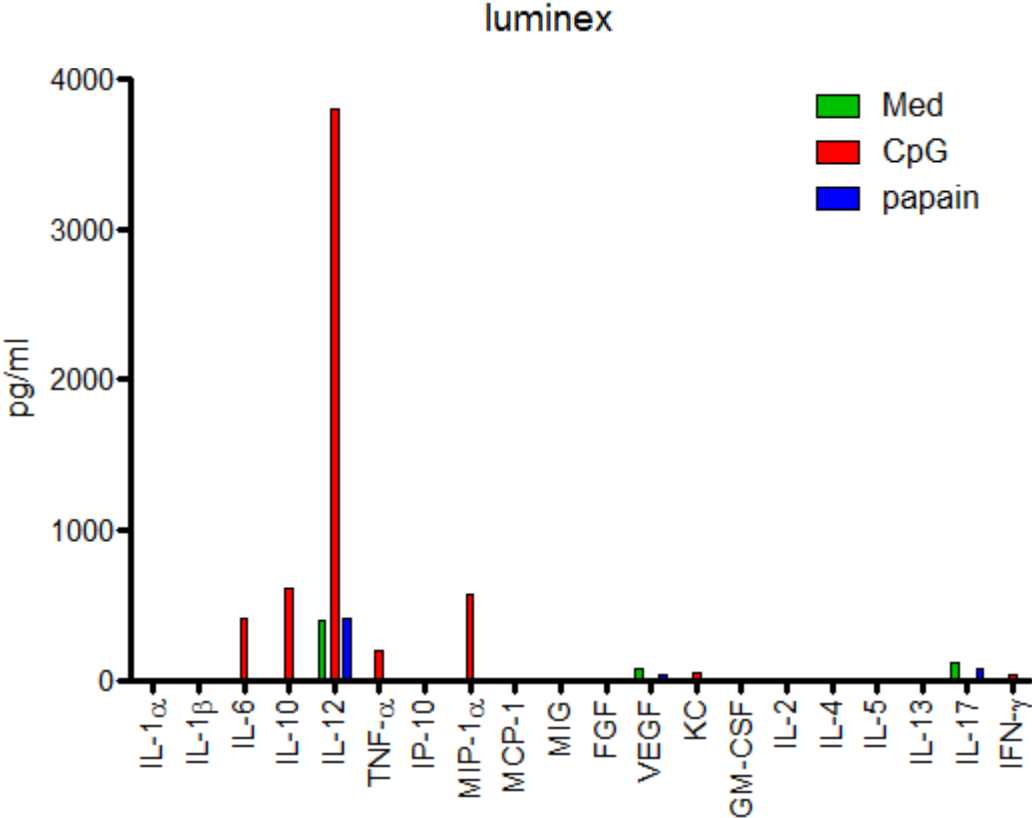
Lymph node DCs or basophils were sorted from mice immunized with papain + OVA, and co-cultured with OT II CD4 T cells ( $1 \times 10^5$ ) in the presence of OVA protein (200  $\mu\text{g/ml}$ ), *in vitro* for 4 days. The proliferation of T cells was examined by <sup>3</sup>H-thymidine incorporation.

S8. Basophils can be efficiently depleted by anti-Fc $\epsilon$ RI $\alpha$  (MAR-1) antibody treatment



Basophils can be efficiently depleted by anti-Fc $\epsilon$ RI $\alpha$  (MAR-1) antibody. C57BL/6 mice were injected twice daily for 3 days with PBS or 5  $\mu$ g anti-Fc $\epsilon$ RI $\alpha$  (MAR-1) antibody. On day 4 the depletion efficiency in blood was examined by FACS.

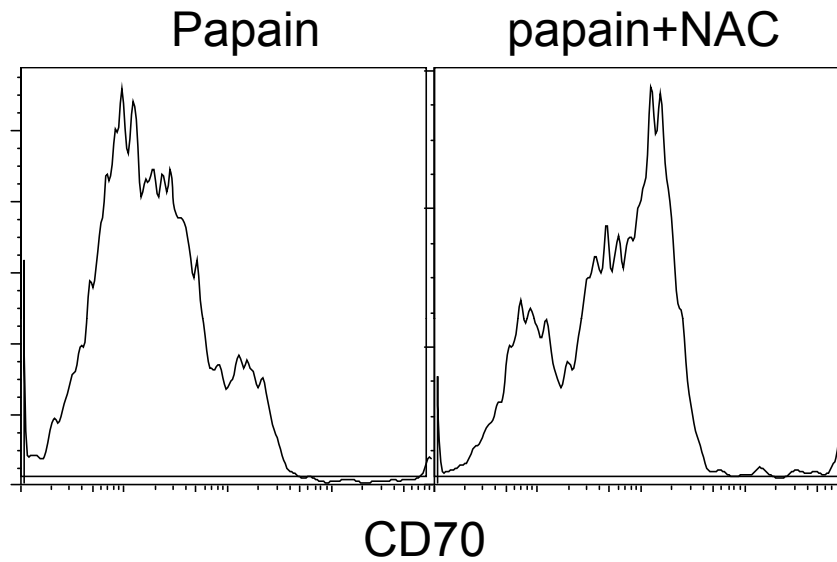
S9. Papain induced production of pro- and anti-inflammatory cytokines and chemokines by lymph node DCs



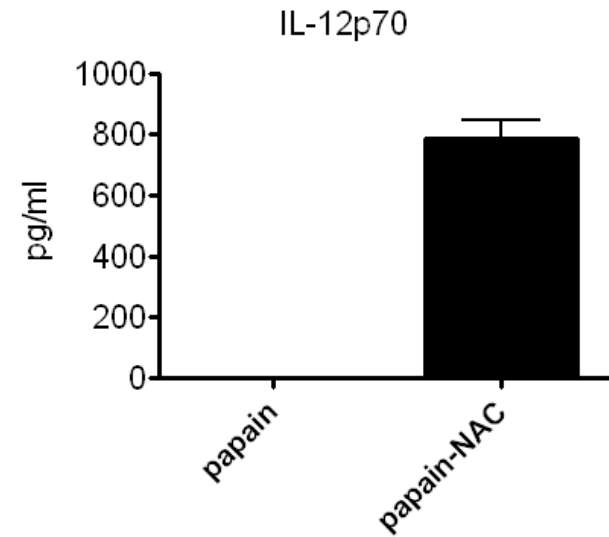
Lymph node DCs were cultured at  $1 \times 10^6/\text{ml}$  and stimulated with CpG ( $1 \mu\text{g}/\text{ml}$ ) or papain ( $25 \mu\text{g}/\text{ml}$ ), or left untreated at  $37^\circ\text{C}$  overnight in the presence of 3T3-CD40L cells ( $5 \times 10^2/\text{ml}$ ). The cytokines in the supernatant were examined by Luminex.

S10. Papain induced ROS suppresses the production of CD70 and IL-12p70 in DCs.

**a**

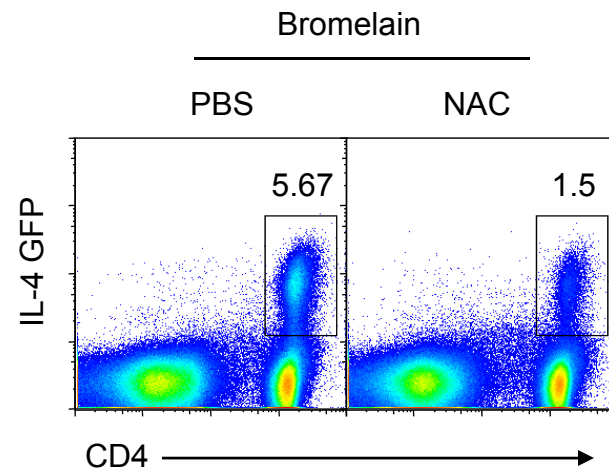


**b**



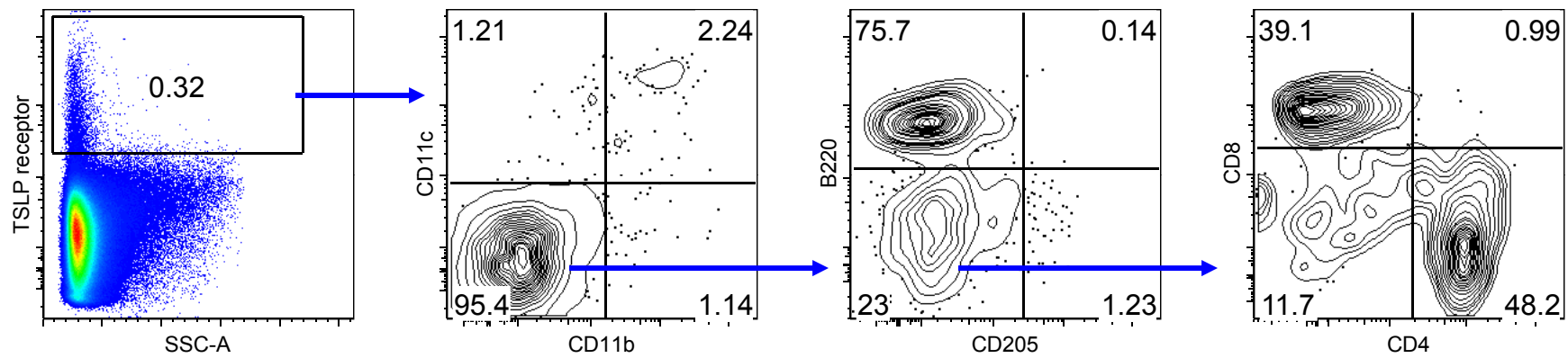
ROS induced by papain suppressed the expression of CD70 and IL-12p70 in DCs. (a) DCs were treated with 25  $\mu$ g/ml papain with or without 6 millimolar NAC *in vitro*, for 18h. CD70 expression was examined by FACS. (b) Papain treated DCs were co-cultured with OT II CD4 T cells in the presence of class II OVA peptide (5  $\mu$ g/ml) and 6 millimolar NAC, or left untreated. IL-12p70 in the culture supernatant at day 1 was examined by ELISA. DC:OT II ratio is 1:10.

## S11. Bromelain induced Th2 response is dependent on ROS



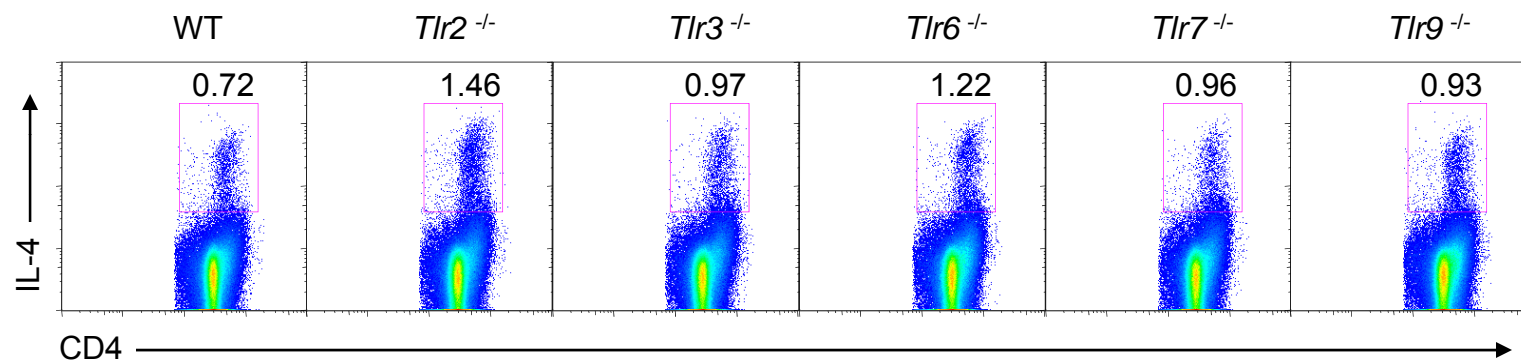
Bromelain-induced Th2 response is dependent on ROS. IL-4-GFP (4get) mice were immunized subcutaneously with bromelain (50  $\mu$ g). Four days later, IL-4 production was detected by FACS. For ROS blocking, mice were injected subcutaneously daily with 150 mg/kg NAC from 2 days prior to immunization to 3 days post immunization.

S12. TSLP receptor is expressed by lymphocytes and DCs in the lymph node



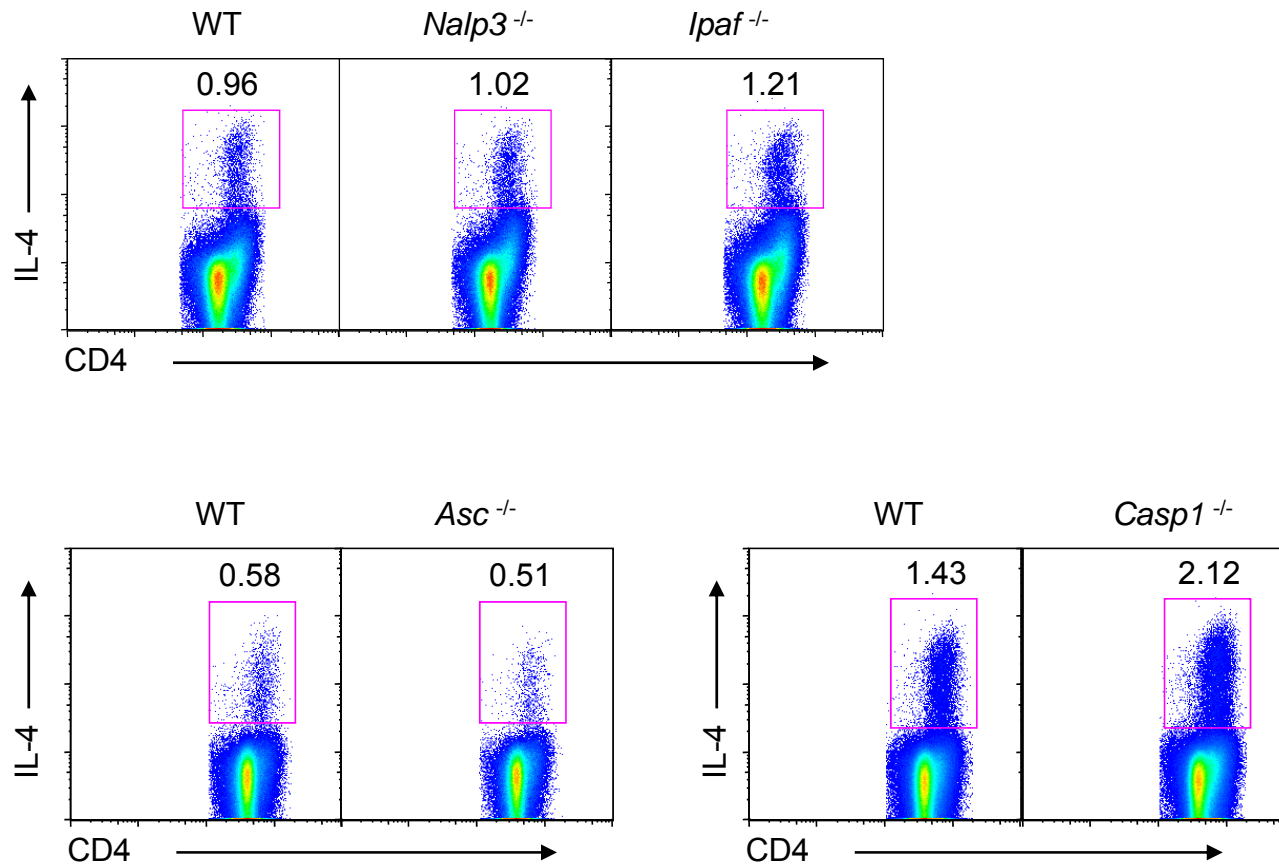
TSLP receptor is expressed by lymphocytes and DCs in the lymph nodes of naive mice. Lymph node cells from naive C57BL/6 mice were stained with the indicated antibodies. The expression of TSLP receptor on each subset was analyzed by FACS.

S13. Papain-induced Th2 response is not dependent of signaling via TLRs 2,3,6,7,9.



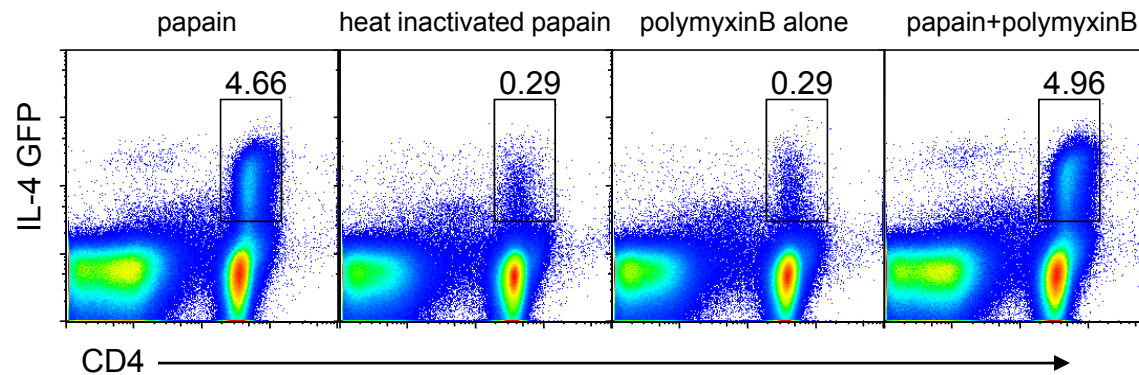
Wild type mice or the indicated knock out mice were subcutaneously immunized with papain + OVA. On day 4 after immunization, draining lymph node cells were isolated and cultured in 96-well plates, pre-coated anti-CD3 (10 µg/ml) and anti-CD28 (2 µg/ml), in the presence of Golgistop for 5 h. IL-4 production was analyzed by intracellular FACS staining.

S14. Papain-induced Th2 response is not dependent on NLR signaling



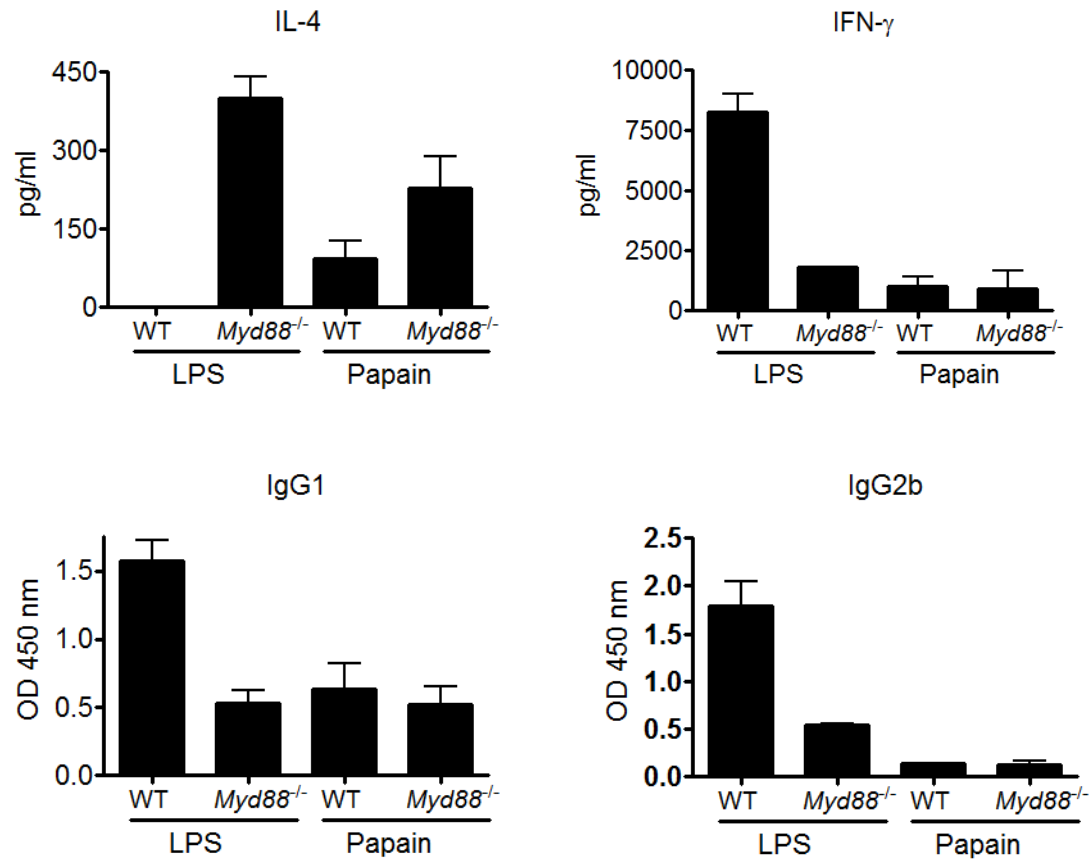
Wild type mice or the indicated knock out mice were subcutaneously immunized with papain + OVA. On day 4 after immunization, draining LNs cells were isolated and in 96-well plates, pre-coated anti-CD3 (10  $\mu$ g/ml) and anti-CD28 (2  $\mu$ g/ml), in the presence of Golgistop for 5 h. IL-4 production was analyzed by intracellular FACS staining.

S15. Papain-induced Th2 response is dependent on the enzymatic activity of papain, and is not inhibited by polymyxin B



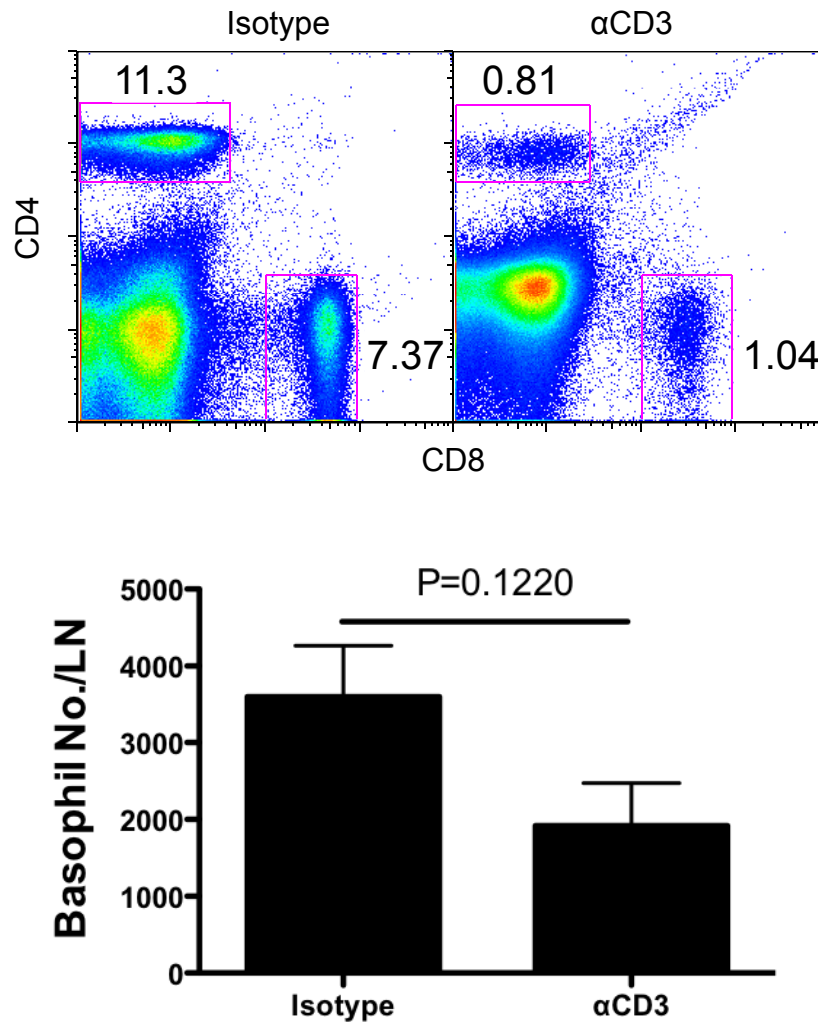
Papain-induced Th2 response is dependent on the enzymatic activity of papain, and is not inhibited by polymyxin B. IL-4-GFP (4get) mice were subcutaneously injected with papain, heat inactivated papain (100 °C, 10 min), polymyxin B, or papain plus polymyxin B. For polymyxin B treatment, mice were subcutaneously injected with 3 mg/kg polymyxin B one day before immunization. On day 4 after immunization, IL-4 production was detected by FACS.

## S16. Papain induced Th2 response is MyD88 independent



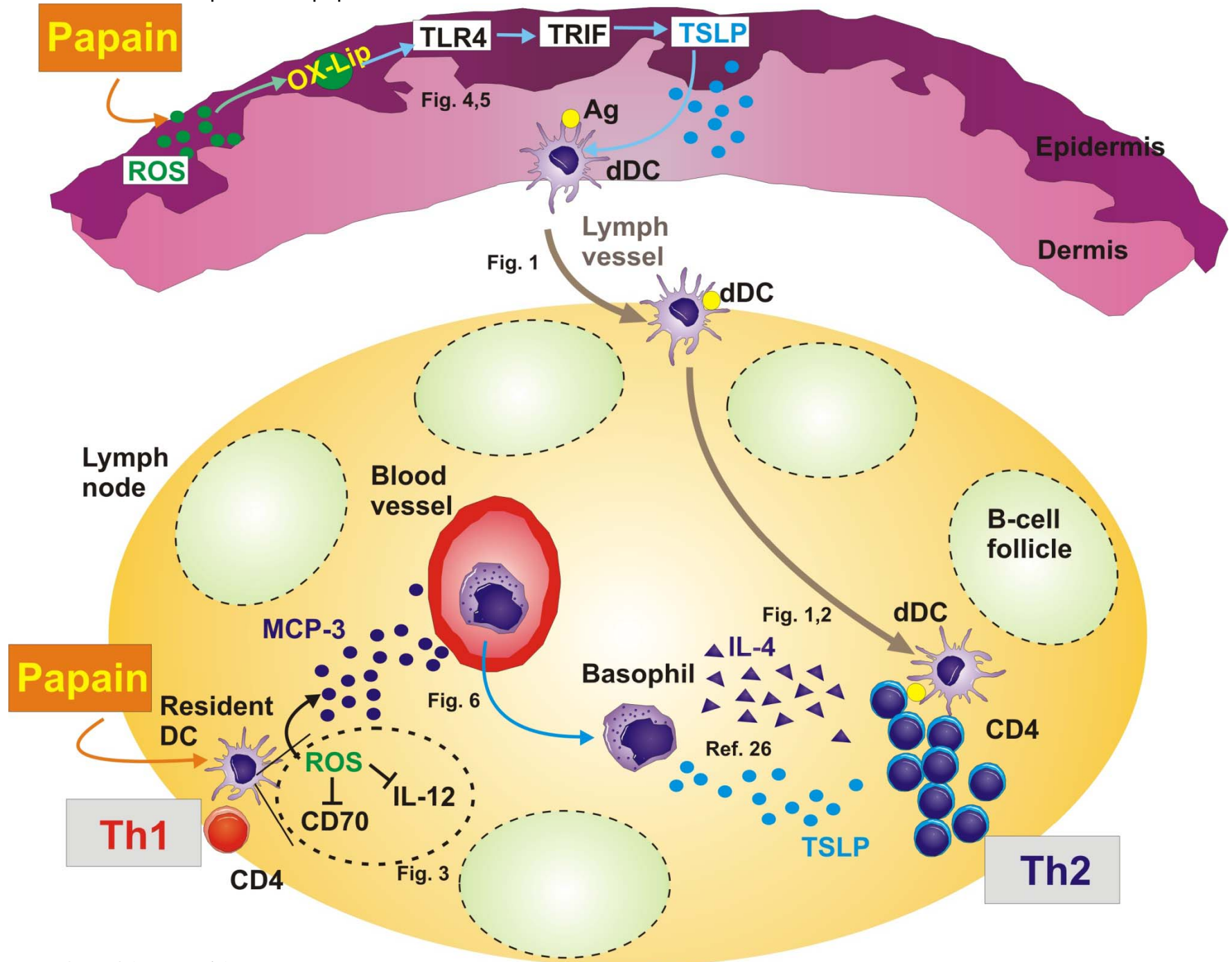
C57BL/6 or *Myd88*<sup>-/-</sup> mice were immunized subcutaneously with papain or LPS (50  $\mu$ g/mouse) plus OVA (100  $\mu$ g/mouse), and repeatedly boosted on day 7 and day 14. On day 21 after the immunization, the draining lymph nodes were removed and total lymph node cells restimulated with OVA in vitro for 5 days, and the cytokines in the supernatants were detected by ELISA. For antibody responses, serum were collected on day 14 and OVA specific antibody isotypes were analyzed by ELISA.

S17. Depletion of T cells does not significantly affect papain-induced basophil migration

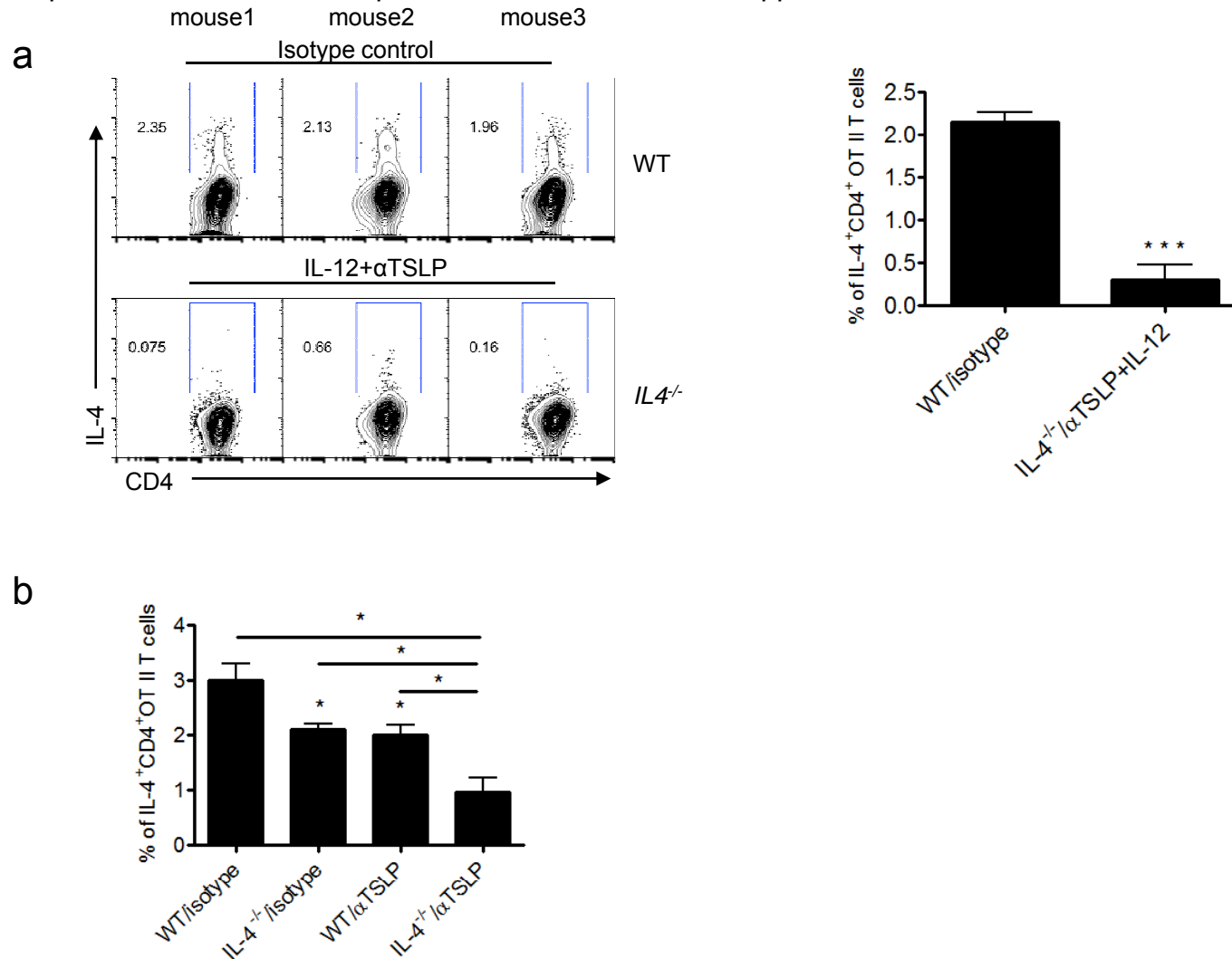


Mice were given intravenous injections of anti-CD3 antibody at a dose of 40  $\mu$ g on day -5, and subsequent daily injections for 7 additional days. On day 0, mice were immunized with papain + OVA. The depletion of T cells in blood was analyzed by FACS on day 0 (upper panel). On day 3 after immunization, draining LNs cells were isolated and the number of basophils was analyzed by FACS.

S18. A model for Th2 responses to papain



# S19. Optimal Th2 induction is dependent on IL-4, TSLP and suppression of IL-12



Wild type mice or *IL4*<sup>-/-</sup> mice were reconstituted with 5x10<sup>5</sup> OT II CD4 T cells on -day1. (a) On day 0, 2hr before immunization, *IL4*<sup>-/-</sup> mice were injected with anti-mouse TSLP antibody (subcutaneously, 250 μg/mouse) and recombinant murine IL-12 (intraperitoneally once daily, 500ng/mouse), while wild type mice were given isotype antibody. Then the mice were immunized with papain (50μg) plus OVA (100μg), subcutaneously at the same site. On days 2 and 3, TSLP was injected again. IL-12 was injected again on days 1, 2 and 3. On day 4, the draining lymph node cells were isolated and restimulated in plates pre-coated with anti-CD3 (10 μg/ml) and anti-CD28 (2 μg/ml), in the presence of Golgistop for 5 h. IL-4 production was analyzed by intracellular FACS staining. The graph on the right is the summary of FACS plot data on the left. (b) OT II reconstituted wild type mice or *IL4*<sup>-/-</sup> mice were treated with anti-mouse TSLP or isotype control, and immunized as in (a). IL-4 production was tested by FACS. \*\*\*p<0.001, \*\*p<0.01, and \* p<0.05.